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AB 4-Hydroxyphenylpyruvate dioxygenase (HPPD, EC 1.13.11.27) is an FeII-dependant mononuclear non-heme dioxygenase that converts 4-hydroxyphenylpyruvate (HPP) into **homogentisate** (2,5-dihydroxyphenylacetate, HG). This reaction is the second step of the pathway for tyrosine catabolism that is ubiquitous in aerobic organisms. In mammals, in born mutations in the gene for fumarylacetoacetase (FAA), the enzyme to catalyze the final step in this pathway, can give rise to the disease, type 1 tyrosinemia (T1Y). In humans T1Y leads to cirrhosis and primary cancer of the liver resulting in death typically by the age of five. Our objective is to alleviate the symptoms of T1Y by specifically **inhibiting HPPD** and hence shutting down the tyrosine catabolism pathway. Our encompassing objective is to characterize the reaction coordinate of HPPD in sufficient detail to identify reasonable transition state geometries for the design of transition state analogs. Initially we have over expressed HPPD from Streptomyces avermitilis to 35% of total cell protein in Escherichia coli. The enzyme in its apo- or holo- forms can readily be isolated using a combination of anion exchange, size exclusion chromatog. The purified oxidized holoenzyme has a weak absorbance band at 600 nm. Under reducing conditions in atm. oxygen the enzyme has an apparent turnover no. of 4 s-1 and exhibits significant substrate inhibition. The substrate, HPP, has keto and enol tautomeric forms of which HPPD exclusively uses the keto form as a substrate. The rate of tautomerization from enol to keto forms is general base catalyzed and HPLC anal. of the mixt. at equil. in aq. soln. shows that the substrate exists as a mixt. of at least eight components. Inhibition studies indicate that a variety of divalent metals compete with iron for occupancy of the active site. Steady state measurements show a Km for HPP under atm. conditions of 27 .mu.M and a Ki of 590 .mu.M. The Km for oxygen is 10.1 .mu.M while the apparent Kd for FeII measured by its steady state dependence is 0.5 .mu.M.

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TI Preliminary studies of 4-hydroxyphenylpyruvate dioxygenase from Streptomyces avermitilis

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